BCMB 3100: Partial notes
Chapter 4 (Part 1)

• Diversity of proteins
• 3D structure of proteins
• Fibrous vs globular proteins
• Conformation vs configuration
• 1°, 2°, 3° and 4° structure
• Peptide groups in polypeptide
• $\phi$ vs $\psi$ angles, Ramachandran plot
• Xray crystallography & NMR
• $\alpha$ helix vs $\beta$-sheet

Diversity of proteins

• _______________ - study of large sets of proteins, such as the entire complement of proteins produced by a cell
• E. coli has about _______ different polypeptides (average size 300 amino acids, M, 33,000)
• Fruit fly (Drosophila melanogaster) about 16,000, humans, other mammals about 40,000 different polypeptides

E. coli proteins on 2D gel electrophoresis

See Page 76

E. coli expresses ~4000 proteins

3D STRUCTURE OF PROTEINS

Two classes of proteins:

_____________ : water insoluble, static, "tough", extended, provide mechanical support ($\alpha$-keratin, collagen)

_____________ : compact, "spherical", usually: hydrophobic interior & hydrophilic exterior enzymes
The biological activity of a protein depends on its conformation

Conformation: spatial arrangement of substituent groups that are free to assume different positions in space, without breaking any bonds, because of the freedom of bond rotation

The number of potential conformations of a protein is _______. Under physiological conditions, the protein assumes a single stable shape: native conformation

Native conformation: a spatial arrangement of atoms that can not be changed without breaking covalent bonds

Levels of Protein Structure

Primary structure: the covalent backbone of a polymer

Secondary structure: the residue-by-residue conformation of the backbone of a polymer

Tertiary structure: the 3D conformation of a polymer in its native folded state

Quaternary structure: the 3D structure of a multisubunit, particularly the manner in which the subunits fit together

Supersecondary structure: clusters of secondary structure (e.g. βαβ)

Domain: a distinct structural unit of a polypeptide; domains have separate functions and may fold as independent, compact units
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Conformation of the Peptide Group:
The peptide group consists of 6 atoms

Resonance structure of the peptide bond:

(a) Peptide bond shown as a C-N single bond
(b) Peptide bond shown as a double bond
(c) Actual structure is a hybrid of the two resonance forms. Electrons are delocalized over three atoms: O, C, N
(d) The peptide bond is planar with partial double bond characteristics. This prevents rotation of the peptide bond and constrains the conformation of the peptide backbone.

Planar peptide groups in a polypeptide chain:

- Rotation around C-N bond is restricted due to the double-bond nature of the resonance hybrid form
- Peptide groups (blue planes) are therefore planar

See Fig. 4.8 Trans and cis conformations of a peptide group:

Nearly all peptide groups in proteins are in the trans conformation

- __________ conformation is less favorable than trans due to steric interference of \( \alpha \)-carbon side chains
- __________ conformation is established protein during synthesis
Rotation of atoms in a peptide group

- Peptide group has a repeating N-C\(_\alpha\)-C backbone
- Rotation about both the N-C\(_\alpha\) (\(\phi\)) and C\(_\alpha\)-C (\(\Psi\)) bonds is possible
- Rotation of the N-C\(_\alpha\) bond in proline is restricted because of the pyrrolidine ring structure
(a) See Fig. 10 (a) Ramachandran Plot

(a) Solid lines: range of permissible $\phi$ and $\psi$ values
Dashed lines: outer limits for an alanine residue
Blue dots: values for known conformations

(b) Ramachandran Plot

• Observed $\phi$ and $\psi$ values in known structures. Crosses are typical values for a single protein.
• $\alpha$-Helix residues (red), $\beta$-Strand residues (blue), others (green).

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____________________: technique that reveals 3D position of atoms in a protein.
1. Need crystallized protein
2. Source of ________
X-ray strikes protein & & some are scattered by electrons in protein.
3.Scattering is detected by X-ray film or solid state electronic detector.
4. Pattern of recombinant waves depends upon atomic structure.
5. Pattern converted to atomic image by computer analysis.
Methods for Determining Protein Structure

- X-ray crystallography is used to determine the three-dimensional conformation of proteins.

Ribonuclease A

(a) Space-filling model (bound substrate analog black)
(b) Cartoon ribbon model (shows secondary structure)
(c) Substrate-binding site view

NMR determination of protein structure

- A method used to determine structure of small proteins in solution. Nuclear magnetic resonance (NMR) is a physical phenomenon in which nuclei in a magnetic field absorb and re-emit electromagnetic radiation.

Ribonuclease A determined by NMR (polypeptide chain backbone)

Protein structures are deposited in the databases: e.g., Protein Data Bank (PDB)

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Secondary structure in proteins

Linus Pauling & Robert Corey (1950s)

proposed _______ and _______ as types of structures in proteins

right-handed
carbonyl O of aa residue n is H-bonded to \( \alpha \)-amino N of residue n+4

rise = 0.15nm,  pitch = 0.54,  3.6 residue per turn

Fibrous proteins (e.g. keratin) may be largely \( \alpha \)-helix

Globular proteins vary greatly in \( \alpha \)-helix content: average \( \alpha \) helix content of 26%

All side chains point outward from cylinder of helix

Ala often found in \( \alpha \) helix;  Pro & Gly usually not present in helix but may be at ends

See Fig. 4.11

The \( \alpha \)-helix

See Fig. 4.11 Stereo view of right-handed \( \alpha \) helix

- All side chains project outward from helix axis
Helix in horse liver alcohol dehydrogenase

(a) Amino acid sequence, (b) Helical wheel diagram

Horse liver alcohol dehydrogenase

- Amphipathic \( \alpha \) helix (blue ribbon)
- Hydrophobic residues (blue) directed inward, hydrophilic (red) outward

This is an example of an amphipathic \( \alpha \) helix

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Fig. 4.14 Leucine zipper of yeast

(See Fig. 4.21)

- DNA binding region consists of two amphipathic \( \alpha \) helices, one from each of two protein subunits.

This transcription factor has amphipathic helices known as a leucine zipper. Leucine zippers have only a lose coiled structure compared to other coiled-coil amphipathic helices.

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\( \beta \) sheet: extended polypeptide strands (\( \beta \) strands) stabilized by H-bonds between carbonyl O and amide H.

Each amino acid accounts for 0.32-0.34 nm of length of polypeptide chain.

\( \beta \) strands may be parallel or antiparallel

\( \beta \) sheets contain 2-15 strands with average of 6 aa residues per strand

\( \beta \) strand content of globular proteins is variable:
Average of 19\% \( \beta \) structure
**Interactions of β sheets**

- β Sheet side chains project **alternately above and below** the plane of the β strands.
- One surface of a β sheet may consist of hydrophobic side chains that can interact with other hydrophobic residues in protein interior.
- __________ β sheets have hydrophobic side chains projecting outward that can interact with hydrophobic faces of β sheets or other helices.

**Structure of PHL P2 protein**  
(grass allergen)

(a) Blue/purple antiparallel β-sheets within a protein  
(b) Stereo view of the β sandwich. Polar residues (red), hydrophobic residues (blue)

*Example of amphipathic interaction in a β sheet*
Loops and Turns (1)

• Loops and turns connect $\alpha$ helices and $\beta$ strands and allow a peptide chain to fold back on itself to make a compact structure
• _______- often contain hydrophilic residues and are found on protein surfaces
• _______- loops containing 5 residues or less
• ___________________ - connect different antiparallel $\beta$ strands (also called hairpin loop)

Loops and Turns (2)

______: 4 aa, stabilized by H-bonds between $\alpha$-carbonyl O of residue n and $\alpha$-NH of residue (n+3); Pro is often 2nd residue

____________: (also called glycine turn, $\beta$ bend) like Type I Turn but 3rd residue is Gly

Reverse turns

(a) Type I, and (b) Type II

See Fig. 4.20

BCMB 3100 Partial Lecture Notes for Chapter 4 (Part 2)

• Supersecondary Structures (Motifs), Domains, Folds, Quaternary Structure
• Anfinsen’s Experiment denaturation, reduction & refolding
• Protein folding, Chaperones
• Collagen, Myoglobin & Hemoglobin
Common motifs

Motifs - recurring protein structures

- independently folded, compact, distinct structural unit in proteins
- ~25 to ~300 amino acid residues
- connected to each other by loops, bound by weak interactions between side chains
- may have separate functions
- illustrate evolutionary conservation of protein structure

Pyruvate Kinase

- Main polypeptide chain folds into three distinct domains
- Last enzyme in glycolysis

Cytochrome c

- Conservation of cyt c structure

(a) Tuna (+heme)
(b) Tuna
(c) Rice
(d) Yeast
(e) Bacteria

See Last slides of Chapter 3 Lecture for Phylogenic Tree
Structural similarity of (a) lactate dehydrogenase, (b) malate dehydrogenase (only 23% identical amino acids!!)

\[
\begin{align*}
\text{CH}_3\text{-HOCH-COO}^- & \quad \text{Lactate} \\
\text{COO}^-\text{-CH}_2\text{-HOCH-COO}^- & \quad \text{Malate}
\end{align*}
\]

(a) \textit{B. stereothermophilus}, (b) \textit{E. coli}

Four categories of protein domains

1. **All \( \alpha \)** - almost entirely \( \alpha \) helices and loops
2. **All \( \beta \)** - only \( \beta \) sheets and non-repetitive structures that link the \( \beta \) strands
3. **Mixed \( \alpha/\beta \)** - alternate regions of \( \alpha \) helix and \( \beta \) strand (e.g. \( \alpha\beta\alpha \) motif)
4. **\( \alpha + \beta \)** - local clusters of \( \alpha \) helices and \( \beta \) sheet in separate, contiguous regions of the polypeptide chain

Folds

- A “fold” is a combination of secondary structures that form the core of a domain
- Some domains have simple folds, others have more complex folds

Common domain folds
Examples of tertiary structure

(a) Human serum albumin
(b) E. coli cytochrome b\textsubscript{562}

(g) Jellyfish green fluorescent protein
(h) Pig retinol-binding protein

Tertiary Protein Structures (cont)

Domain Structure and Function

- A _____________ may have a particular function
- ___________ between 2 domains provide crevices, grooves, and pockets on the surface of a protein for binding or catalytic sites
- In multifunctional enzymes, each catalytic activity can be on one of several domains

Quaternary Structure

- organization of subunits in a protein with multiple subunits (an "oligomer")
- Subunits (may be identical (\(\alpha\alpha\)) or different (\(\alpha\beta\)) have a defined stoichiometry and arrangement
- Subunits are held together by many weak, noncovalent interactions (hydrophobic, electrostatic)
Quaternary structure of multidomain proteins

(a) Chicken uridine diphosphate glucose transferase
Subunits = $\alpha_2$

(b) HIV-1 aspartic protease
Subunits = $\alpha_2$

(c) Streptomyces potassium channel precursor
Subunits = $\alpha_4$

(d) Rhodopseudomonas capsid protein
Subunits = $\alpha_3$

(continued)

Protein Denaturation and Renaturation:

- Partial or complete unfolding of native conformation
- Causes loss of biological activity
- Caused by heat, extreme pH, detergents, chaotropic agents; due to disruption of non-covalent interactions
- Some proteins can be refolded or renatured
("chaos-promoting"): chemicals that denature proteins (e.g. urea, guanidinium chloride)

Chaotropic agents do NOT cleave covalent bonds but disrupt 2°, 3° and 4° structure

Chaotropic agents do NOT cleave covalent bonds but disrupt 2°, 3° and 4° structure.

Urea and guanidinium chloride
Two chaotropic agents

Disrupt hydrophobic interactions in interior of protein by disordering water molecular adjacent to the protein.

Chaotropic agents increase entropy of system by interfering with hydrogen bonds, Van der Waals forces, and hydrophobic effects.

Detecting denatured Proteins
• Heat denaturation of ribonuclease A
• Unfolding monitored by changes in ultraviolet (blue), viscosity (red), optical rotation (green)

\[ T_{m} \text{ (melting temperature)} = \text{temperature when 50% of particular protein is denatured} \]

oxidation
\[ \text{R-SH} + \text{HS-R} \rightarrow \text{R-S-S-R} \]

\[ \text{reduction} \]

\[ \text{oxidation: the loss of electrons from a compound} \]

\[ \text{reduction: the gain of electrons by a compound or ion} \]

Common reducing agents: β mercaptoethanol; dithiothreitol
Disulfide bridges in bovine ribonuclease A

Ribonuclease:
- 124 aa,
- mainly β sheet,
- 4 disulfide bonds

(a) Location of disulfide bridges
(b) Stereo view of Cys-26 and Cys-84

The amino acid sequence specifies 3D structure!!

The native form of a protein (e.g., ribonuclease) appears to be the thermodynamically most stable structure

Denaturation and renaturation of ribonuclease A

Protein Folding and stabilization (1)

Cooperativity of folding: formation of one part of structure (e.g., initial aa interactions) leads to formation of remaining structure.

is MAJOR driving force in protein folding
**Protein Folding and stabilization (2)**

Protein folding $\rightarrow$ nonpolar side chains inside, most polar chains outside $\rightarrow$ 2$^\circ$ structure

H-bonds and van der Waals forces stabilize globular protein folding

Covalent cross links (R-S-S-R) and ionic interactions may stabilize some globular proteins

---

**CURRENT THEORY OF PROTEIN FOLDING**

1. not random
2. cooperative and sequential
3. dependent on 1$^\circ$ structure
4. for some proteins 1$^\circ$ structure alone determines folding
5. some proteins require help to fold (e.g. enzymes or chaperones)
6. extremely rapid, native conformation is generally reached $< 1$ second
7. NOTE: most protein have single native 3D shape; but some exceptions

---

*__________:* proteins that bind newly synthesized polypeptides & assist in proper folding

*__________:* increase rate of correct folding and prevent the formation of incorrectly folded intermediates

*__________:* bind unassembled protein subunits, prevent incorrect aggregation before assembly into multisubunit protein

*Most chaperones are heat shock proteins*

---

**E. coli chaperonin**

(a) (b) Core consists of 2 identical rings (7 GroE subunits in each ring)

(c) Protein folding takes place inside the central cavity
Chaperonin-assisted protein folding

- Hydrolysis of several ATP molecules is required

![Unfolded polypeptide](image1) + n ATP → Chaperone → n ADP + n P

![Folded polypeptide](image2)

Collagen Triple Helix (1)

- Most abundant vertebrate protein (25-35% total protein); fibrous; found in bone, tendons, skin, blood vessels, cartilage; gelatin, glue

Collagen is an aggregate of 3 left-handed helices coiled into a right-handed super helix!

- Has high percentage of Pro, Gly, Hyp (4-hydroxyproline),

- Also contains Hyl (hydroxylysine)

- Structure elucidated by G. N. Ramachandran

Collagen Triple Helix (2)

- Hyp & Hyl are formed posttranslationally

- Hyp allows more H-bonding & stabilization of collagen triple helix

- Pro & Hyp prevent formation of α helices & make collagen fibers rigid

- Strengthened by inter- and intra-covalent crosslinks between allysine-Lys (allysine)

- Vitamin C (ascorbate) deficiency → decrease in Hyp & Hyl formation → ________ (weakness in blood vessels & skin)
Ascorbate (vitamin C) is a reducing agent that keeps prolyl hydroxylase in an active form. Primates and guinea pigs have lost ability to synthesize ascorbic acid, so they must obtain it from their diet.

Collagen triple helix (3)
- Multiple repeats of -Gly-X-Y-; X often proline, Y often 4-hydroxyproline
- Glycine residues located along central axis of a triple helix (other residues cannot fit)
- For each -Gly-X-Y- triplet, one interchain H bond forms between amide H of Gly in one chain and -C=O of residue X in an adjacent chain
- No intrachain H bonds exist in the collagen helix

Interchain H bonding in collagen
- Amide H of Gly in one chain is H-bonded to C=O in another chain
Some proteins require **cofactors** or **prosthetic groups** at their active sites for activity

_________ : small, usually nonprotein molecule required for enzyme activity

___________ : a metal ion or other non-amino acid molecule tightly bound to a protein and essential for its activity

___________ : active protein with all its cofactors

___________ : protein without its cofactors

---

**Myoglobin (Mb) [See Chapter 9 !]**

Binds $O_2$
Stores & transports $O_2$ in muscle

**Prosthetic group:** heme = Fe-protoporphyrin IX

Single polypeptide (153 aa) + heme

Globular protein with 8 $\alpha$-helices

Oxygenated myoglobin = oxymyoglobin

All polar residues (except 2 His's) are located on protein surface

---

**Hemoglobin (Hb)**

Binds $O_2$

Transports $O_2$ in vertebrate blood

**Prosthetic group:** heme = Fe-protoporphyrin IX

Tetramer: $\alpha 2\beta 2$; $\alpha$ has 141 aa; $\beta$ has 146 aa; acts as dimer of $\alpha\beta$

$3^\circ$ structure of each subunit similar to myoglobin

---

**Heme Fe(II)-protoporphyrin IX**

- Porphyrin ring provides four of the six ligands surrounding iron atom

The ferrous iron is held in place by binding of nitrogens of the 4 pyrrole rings

See Pg. 151
The structure of globins has been conserved in many species.

Amino acid changes (substitutions) may or may not affect protein conformation.

________ do NOT significantly effect conformation (i.e. Val → Ile)

________ DO effect conformation (Glu → Val)

Sperm whale oxymyoglobin

- Oxygen (red)
- His-93 and His-64 (green)

Hemoglobin tetramer

See Fig. 4.30  See Fig. 9.6

(a) Human oxyhemoglobin  (b) Tetramer schematic

(a)  (b)

Tertiary structure of myoglobin, α-globin and β-globin

α-Globin (blue)
β-Globin (purple)
Myoglobin (green)
Oxygen-binding site of whale oxymyoglobin

- Octahedral geometry of coordination complex (six ligands around iron)
- His-93 (proximal histidine) liganded to Fe
- His-64 (distal histidine)

See Fig. 9.4

Oxygen-binding curves

(a) Comparison of O₂-binding to Mb and Hb

Hyperbolic curve (single equilibrium constant)

\[ \text{Mb} + \text{O}_2 \Leftrightarrow \text{MbO}_2 \]

Fractional Saturation = \( Y = \frac{[\text{MbO}_2]}{[\text{MbO}_2] + [\text{Mb}]} \)

p50 = partial pressure at half saturation

See Figures 9.1 & 9.2

(b) Oxygen-binding curves for observed and for high and low affinity forms of hemoglobin

(b) Binding of the R (high-affinity) and T (low affinity) forms of Hb

Conformational changes in a hemoglobin chain induced by oxygenation

- Oxygen binding to Fe pulls the proximal His toward ring plane
- Helix with His shifts position, disrupting some ion pairs between subunits (blue to red position)

See Fig. 9.7
Enhanced activity resulting from cooperation between subunits of an allosteric protein

A protein having multiple active sites as well as distinct regulatory sites that control the flux of biochemicals through a metabolic pathway.

Hemoglobin is an allosteric protein: regulatory protein whose activity is modulated by noncovalent binding of a specific metabolite at a site other than the active site

allosteric regulation is caused by small changes in native conformation of a protein

active shape = R (relaxed)
inactive shape = T (taut)

allosteric inhibitor
R  \rightarrow  T

allosteric activator

2,3-bisphospho-D-glycerate (2,3BPG) is an allosteric effector of hemoglobin. It lowers the affinity of deoxyhemoglobin for O₂ (raises P50). [Know physiological significance for Exam]

Bohr effect: the increase in P50 of hemoglobin caused by a lowered pH due to an increase in CO₂

2,3-Bisphospho-D-glycerate (2,3BPG)
Binding of 2,3BPG to deoxyhemoglobin

- Charges on 2,3BPG pair with (+) charges lining the central cavity, stabilizing the DeoxyHb form.
- α-Subunits pink, β-subunits blue, heme groups red.

See Fig. 9.10

Bohr effect

- Lowering the pH decreases the affinity of Hb for oxygen.

Antibodies Bind Specific Antigens

- Vertebrate immune systems synthesize protein antibodies (immunoglobulins) to eliminate bacteria, viruses, other foreign substances.
- Antibodies specifically recognize and bind antigens.
- Antibodies are synthesized by lymphocytes (white blood cells).

Fetal hemoglobin has lower binding affinity for 2,3-BPG than maternal hemoglobin. Thus, oxygen affinity of fetal red blood cells is higher than maternal red blood cells.
(a) Human antibody structure
Immunoglobulin G class; IgG) (see Fig. 5.16)

Light Chain contains 2 domains; Heavy Chain contains 4 domains
Each domain: 110 aa in common motif known as immunoglobulin fold

(see Fig. 5.16)

• Heavy chains (blue) and light chains (red)
• Disulfide bonds (yellow)
• Variable domains colored darker

Antibody (IgG) Structure and their use in characterizing and purifying proteins

Both Polyclonal and Monoclonal antibodies can be used to characterize proteins.

Both can be used for Western Blotting and ELISAs and “immunoprecipitation” (in the broadest sense).

Fig. 5.16

Fig. 5.18
Purification of proteins by “immunoprecipitation”

Antibody is attached to insoluble and/or magnetic bead

Fig. 5.21

Enzyme-linked immunosorbent assay (ELISA)
Indirect and Direct ELISAs

(A) Indirect ELISA
Antigen-coated wall
Specific antibody binds to antigen
Enzyme-linked antibody binds to specific antibody
Substrate is added and converted by enzyme into colored product; the amount of color formation is proportional to the amount of specific antibody

(B) Sandwich ELISA
Monoclonal antibody-coated wall
Antigen binds to antibody
A second monoclonal antibody, linked to enzyme, binds to immobilized antigen
Substrate is added and converted by enzyme into colored product; the amount of color formation is proportional to the amount of antigen

Fig. 5.22

Western Blotting

Fig. 5.23

The following are additional notes that will help you in your studying
**Review of Globular Protein 3D Structure**

Most globular proteins have a compact globular shape due to many reversible turns in direction combined with the α helix and/or β structure. Usually, hydrophobic amino acid residues are in the interior and hydrophilic amino acid residues on the exterior of the protein.

The loops & turns contain nonrepetitive regions of 2° structure.

- **loops**: range from ~ 2-16 residues, many hydrophilic residues found at the surface of the protein (can H-bond with water)
- **turn**: loops having only a few residues (<6)

**Average structure of Globular Protein**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>α helix</td>
<td>26%</td>
</tr>
<tr>
<td>β structure</td>
<td>19%</td>
</tr>
<tr>
<td>Turns</td>
<td>15%</td>
</tr>
<tr>
<td>Simple loops</td>
<td>21%</td>
</tr>
<tr>
<td>Complex loops</td>
<td>10%</td>
</tr>
</tbody>
</table>

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**Four categories of protein domains**

(1) **All α** - domains almost entirely α helices and loops

(2) **All β** - domains contain only β sheets and non-repetitive structures that link the β strands

(3) **Mixed α/β** - supersecondary structures where regions of α helix and β strand alternate (e.g. αβα motif)

(4) **α + β** - local clusters of α helices and β sheet in separate, contiguous regions of the polypeptide chain

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**Protein Denaturation and Renaturation**

- **Denaturation** - disruption of native conformation of a protein, with loss of biological activity
- Most denatured proteins adopt a random-coil conformation
- Proteins denatured by heating or chemicals
- Some proteins can be refolded or renatured

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**Protein Folding and Stabilization (1)**

- **Cooperativity of folding**: formation of one part of structure (e.g. initial aa interactions) leads to formation of remaining structure.
- **Hydrophobic effect** is MAJOR driving force in protein folding

Folded proteins occupy a low-energy well that makes the native structure most stable

Many proteins can fold spontaneously to this low-energy conformation

Proteins are thought to fold “cooperatively” ... the first few interactions assist subsequent alignment and folding
Protein folding (more detail)

- extremely rapid, native conformation is generally reached $< 1$ second
- During folding the polypeptide collapses in upon itself due to the hydrophobic effect
- An intermediate “molten globule” forms with elements of secondary structure
- The backbone is rearranged to achieve a stable native conformation

Chaperones:
- proteins that bind newly synthesized polypeptides & assist in proper folding
- Chaperones increase rate of correct folding and prevent the formation of incorrectly folded intermediates
- Chaperones bind to unassembled protein subunits to prevent incorrect aggregation before they are assembled into a multisubunit protein
- Most chaperones are heat shock proteins (synthesized as temperature increases)

Two conformations of hemoglobin: T and R
- Active (R state) and inactive (T state) forms are in rapid equilibrium in allosteric proteins
- Binding of substrates and allosteric activators stabilize the R state and shift the equilibrium in the R direction
- Allosteric inhibitors stabilize the T state and shift the equilibrium in the T direction